ABSTRACT

Roselle calyx is rich with phenolic substances. As an antioxidant and antibacterial agent, this substance is not resistant to the change of environment. The alternative way to solve this situation was by using nanoencapsulation technique, to protect bioactive compound using maltodextrin as matrix agent. In this research spray drier was used to dry the nanocapsule from Roselle calyx. Response surface methodology was used to determine the optimum condition. The effects of concentration of nanocapsule agent, compression and temperature on total phenols yield were investigated. The result of study was the optimum condition of spray drier in nanoencapsulation technique was: 120°C temperature, 3 bar of compression and 10% of concentration of nanocapsule. Inhibitory zone was mm against E. coli and mm against S. aureus.

Keywords: Roselle calyx, Response surface method, spray drier, Phenols

INTRODUCTION

Recently, the increasing of food industries have impact to the used of additive food such colourant and preservative which is not safe to the health of consumer in long term but profitable to producer. On the other side consumer awareness for the replacing of synthetic preservative to natural one was increasing. This is opportunity for natural additive food especially which is from plant.

Roselle (Hibiscus sabdariffa) is also known as rosella in Indonesia. Initially, it is cultivated in this region for its fiber, but now also for leaf, fleshy calyx and seed according to their respective properties. The thick red and fleshy cup shaped calyx of the flower are consumed worldwide as a cold beverage and as warm tea. This plant is also used in traditional medicine against many complaints that including high blood pressure, liver diseases and fever (Tsai et al., 2004).

As a natural agent alternative, bioactive compounds of roselle calyx have to extract for more flexible uses in food industry. According to Purbowati et al. (2016), extraction method assisted with microwave power has been proved more efficient for extracting the bioactive compounds of roselle calyx. Roselle extracted by microwave assisted extraction was done at this condition: 250...
Wattof microwave power, for 4,91 minutes, and 78,36 % ethanol as solvent. The roselle extract was containing bioactive compounds total phenolic, anthocyanin and vit C respectively are: 23.77 ± 0.25; 14.80 ± 0.08; 10.74 ± 0.14 mg/g. These extract also has antioxidant and antibacterial activity. According to Pietta (2000), dried calyces contain the flavonoid gossypetin, sabdaretin, hibiscetin and anthocyanin. Mourtzinos et al. (2008) stated that the calyx of Roselle was rich in phenolic compounds including anthocyanin. It was reported that those compounds could be considered as a great source of natural antioxidants.

Unfortunately, this liquid extract has disadvantages such as unstable in environment changes, limited used in food industries and difficulties in material handling and transportation. Based on these reasons, it is necessary for protecting the activity of bioactive compounds in roselle calyx by nanoencapsulation technique. The aim of this technology is to protect the core from external influences in nano size.

Purbowati et al. (2016) stated that the extract of Roselle calyx was rich in phenolic compounds including anthocyanin. It was reported that those compounds could be considered as a great source of natural antioxidants. As anthocyanin are derivatives of the basic flavylium cation structure, which has an electron deficient nucleus, they generally are highly reactive. This reactivity shows the capabilities of anthocyanin as antioxidant. In the other hand, this reaction usually involves decolorization on the pigment. The rate of anthocyanin destruction depends on many factors such as pH, temperature, intermolecular copigmentation, ascorbic acid, oxygen, etc.

Based on the dependent characteristic of phenols, and the need to find the most efficient nanoencapsulation technique have emphasized the need of optimizing the nanoencapsulation process. The Response Surface Method (RSM) is a collection of mathematical and statistical technique for the investigation and modeling of complex problem processes whose response of interest is influenced by several variables and objective to optimize this response (Montgomery, 2001). RSM takes interactions into consideration and optimizes the process parameters to reasonable range, with the advantage of less the number of replicates and the total time required to perform the experiments (Lee et al., 2006). RSM uses an experimental design such as the central composite design (CCD) to fit a model by least squares technique. There has been no report on optimizing nanoencapsulation of phenols from roselle using RSM technique. The objectives of the work were to establish an optimised condition of nanoencapsulation for roselle calyx phenols, and to characterize of the nanocapsule of the optimum condition.

MATERIAL AND METHODS

1. Material

Roselle calyces were bought from Beringharjo Market, Yogyakarta Indonesia.
Solvent used in this experience was ethanol (pa). *Staphylococcus aureus* and *Escherichia coli* were bought from Biology faculty General Soedirman University. Encapsulant agent used was Maltodextrins.

2. Extraction

The dried Roselle calyces were ground for 1 minute using grinder and filtered in 60 mesh. The extraction with concentrations of ethanol 78.36%, microwave power extraction 250 Watt and time of extraction 4.91 minutes were conducted in the ratio dried Roselle and solvent 1:10 w/v, 10 gram dried Roselle powder, in 100 mL solvent (Purbowati *et al*., 2016). The suspension was radiated in microwave oven at regular intervals (one minute radiation and two minute off) to keep the temperature not rise above the boiling point (Li *et al*., 2009). Roselle extract was filtered and concentrated with vacuum evaporator at 70°C 44 cmHg.

3. Nanoencapsulation

Nanoencapsulation process was begun with nanoencapsulation solution processing. Maltodextrin mixed with aquadest with ratio of nanoencapsulant agent (g): water (mL) =1:5 stirred for 30 minutes. Roselle concentrate was added to solution with ratio of solution : extract = 20 : 1. These solution was homogenised at 40°C, 30 minutes. Reduction size to nanoparticles with Tokebi 22,000 rpm for 5 minutes, and dried with spray drier (Naufalin dan Rukmini, 2013).

4. Sample Preparation

Sample was prepared by adding to 100 mg nanocapsules, 4 mL etanol 70%. Stirred these solution at 200 rpm for 2 hrs. After that this solution was sentrifused for 15 minutes at 1000 rpm. These Supernatan was used for the next evaluation. The main response parameters that analyzed were total phenols. The best combination was also analyzed vitamin C, (AOAC, 2000) and total anthocyanin content (Fulekiand Francis, 1968).

5. Determination of total phenols

Total phenols were determined by the Folin-Ciocalteu method of Chew *et al*. (2009). Briefly, 1.5 mL Folin-Ciocalteu reagent (10% v/v) was mixed with 1.5 mL 7.5% (w/v) Na$_2$CO$_3$ solution, then 0.4 mL sample solution was added. After a 90-min incubation at room temperature in dark, the absorbance was measured at 765 nm using Spectrofotometer UV Vis 1800 Shimadzu. Gallic acid was used as a standard compound for the standard curves. The results were presented in mg Gallic acid equivalent (GAE)/g extract.

6. Screening of antibacterial activity (Doughari, 2006)

Screening of antibacterial activity of the plant extract was performed by disc diffusion technique which is highly effective for rapidly growing microorganism. The 20 µL test microorganism were inoculated on to the respective NA medium by pour plate method with 24 hours incubation. After solidification the filter paper disc (6 mm diameter) impregnated with 10 µL crude extract sample, standard antibiotic (Kloramfenicol) and a blank disc impregnated with 10 µL...
respectively, solvent had included. This screening was tested against gram positive bacteria Staphylococcus aureus and gram negative bacteria Escherichia coli. The inoculated plates were incubated for 24 hours at 30°C. The antibacterial activities of the extracts were determined by measuring the clearance zone surrounding the disc.

7. Antioxidant activity (Al Hashimi, 2012)

0.6 mL of sample was dissolved in 0.12 mL of 98% ethanol and 2.88 mL of a 2.51% linoleic acid and 9 mL of a 40 mM phosphate buffer (pH 7.0) were added. The mixture was incubating at 40°C in a test tube in the dark for 3 days (72 hours). After incubation, a 0.1 mL was taken from the mixture and diluted with 9.7 mL of 75% ethanol, followed by the addition of 0.1 mL of 30% ammonium thiocyanate. Precisely three minutes after adding the 0.1 mL of 20 mM ferrous chloride in 3.5% hydrochloride acid, the absorbance of the red color was measured at 500 nm, using Spectrofotometer UV Vis 1800 Shimadzu. The level of lipid peroxidation inhibition by each fraction was calculated from the absorbance ratio to that of a blank without any sample. A half lipid peroxidation inhibition expressed with IC

\[
\text{Antioxidant activity (\%) = } \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%
\]

8. Experimental design

One response was measured: Total phenolic yield (Y), defined as the ratio of total phenols in the extract to total amount of raw material expressed as GAE milligrams per gram of raw material (wet weight). Each of variables to be optimized was coded at 3 levels: -1, 0, and 1. A Central Composite Design (CCD), was arranged to allow for fitting of a second-order model. The model proposed for the response (Y) was:

\[Y = \beta_0 + \beta_1 x_1 + \beta_2 x_1^2 + \beta_{11} x_1 x_1 + \epsilon\]

Where \( \beta \) was the value of the fitted response at the center point of the design, which is point (0, 0, 0). \( \alpha, \beta, \) and \( \epsilon \) were the constant, linear, quadratic and cross-product regression terms, respectively.

RESULT AND DISCUSSION

Optimization of MAE condition of phenol

This research evaluated 3 parameters, which are: (A) Temperature, (B) compression, and (C) concentration. There were three levels for each parameter and second order model used, shown in Table 1.

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In order to reduce the number of experiments, a Central Composite Design was used (Table 2). In this way, only 20 experiments were necessary.

Table 4. Matrix of Central Composite Design

<table>
<thead>
<tr>
<th>Unit</th>
<th>A (°C)</th>
<th>B (Bar)</th>
<th>C (%)</th>
<th>Total Phenol (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>130</td>
<td>4</td>
<td>20</td>
<td>8,7967</td>
</tr>
<tr>
<td>2</td>
<td>130</td>
<td>4</td>
<td>3,18</td>
<td>12,3442</td>
</tr>
<tr>
<td>3</td>
<td>146.82</td>
<td>4</td>
<td>20</td>
<td>10,8661</td>
</tr>
<tr>
<td>4</td>
<td>130</td>
<td>2.32</td>
<td>20</td>
<td>15,0893</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>3</td>
<td>30</td>
<td>7,7198</td>
</tr>
<tr>
<td>6</td>
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<td>4</td>
<td>20</td>
<td>10,6971</td>
</tr>
<tr>
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<tr>
<td>9</td>
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<td>20</td>
<td>130</td>
<td>4</td>
<td>20</td>
<td>9,8947</td>
</tr>
</tbody>
</table>

Multiple regression analysis is:
Total Phenol (Y) = +10.63 - 0.82A - 0.65B - 1.96C + 0.21AB + 0.62AC + 0.70BC + 0.49ABC

The result of ANOVA is shown in Table 3. The Model F-value of 3.11 implies the model is significant. There is only a 4.06% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case C (Concentration) are significant model terms. Values greater than 0.1000 indicate the model terms, temperature and compression, are not significant. The coefficient of determination (R-Squared) is the proportion of variability in the data explained or accounted for by the model. The "R-Squared" of 0.6449 is desirable, mean that the model was fitted for this case. There was no interaction between terms in this research has significantly different influences.

Table 5. ANOVA for all codified term

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F Value</th>
<th>p-value</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>76,6432</td>
<td>7</td>
<td>10,9490</td>
<td>3.1134</td>
<td>0.0406</td>
<td>significant</td>
</tr>
<tr>
<td>A-Suhu</td>
<td>9,2830</td>
<td>1</td>
<td>9,2830</td>
<td>2.6397</td>
<td>0.1302</td>
<td></td>
</tr>
</tbody>
</table>
Optimization is the process to find out the variables value which are optimal, effective and efficient according the target response. In this research the target response was the highest total phenols. Figures 1 shows three dimensionals response surface was presented for the independent variables (temperature, pressure and concentration) which were obtained by keeping another variable constant. The optimum condition of spray drier in nanoencapsulation technique was: 120°C temperature, 3 bar of compression and 10% of concentration of nanoencapsulant. The figure indicate the changes in total phenol yield under different Spray drier conditions. RSM shows only concentration variable was significantly different. In this research Maltodextrin was used as nanoencapsulant agent. Maltodextrin is the substance which is can soluble in water and can protect Phenol substances against deterioration.

Figure 1. Three dimensionals response surface of total phenol

According to Ahmed et al. (2009), maltodextrin could protect the phenol substance during the violet potato powder processing. Rosenberg et al. (1990) stated that the solid concentration would influence the viscosities and play important role in volatile compound dissapereance. this research shows that the concentration 10,03% of nanoencapsulant agent was the optimal concentration of maltodextrin, in temperature 120,01°C and 3,05 compression. The increasing of the nanoencapsulant agent did not increased the total phenols. In higher concentration, the viscosities of liquid more rigid and will influence the percentage of phenol.
entrapment. This caused by the agitation could not force the phenol to come in to matrix.

In 10% of concentration, all bioactive compound which is in matrix could protect from deterioration effect from enviroment changes. in higher concentration of nanoencapsulan, beside the viscousity could make the agitation would not force the phenol to come in to the matrix, total phenol which is still outside the matrix deterioted by the higher temperature of spraydrier.

Verification

The experiment to verified the optimum condition was done in 3 replicates. The result of the verification experiment different from the estimation because the spraydrier could not reset on 120,03⁰C and 3,05 bar of compression. It done in 120⁰C and 3 bar of compression

Profil morfologi nanocapsule

![Figure 2. Size of the roselle nanoencapsulation](image)

The figure show that The particle size which was produced from nanoencapsulation processing using spray drier was under 20 um. These analyse was using SEM using 1000X. Nanoencapsukation using spray drier was the efective way to protect bioactive compound from deterioration and volatile losses (Tonnon et al., 2010; Zaens et al., 2009).

CONCLUSION

1. The optimum condition of spray drier in nanoencapsulation technique was: 120⁰C temperature, 3 bar of compression and 10% of concentration of nanoencapsulan.
2. The antibacterial activity is shown by the inhibition zone formed mm against E coli and S aureus mm against.

REFERENCE


Fuleki T. Francis FJ. 1968. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in Cranberries, *J. Food Sci.* 33(1); 72-77


